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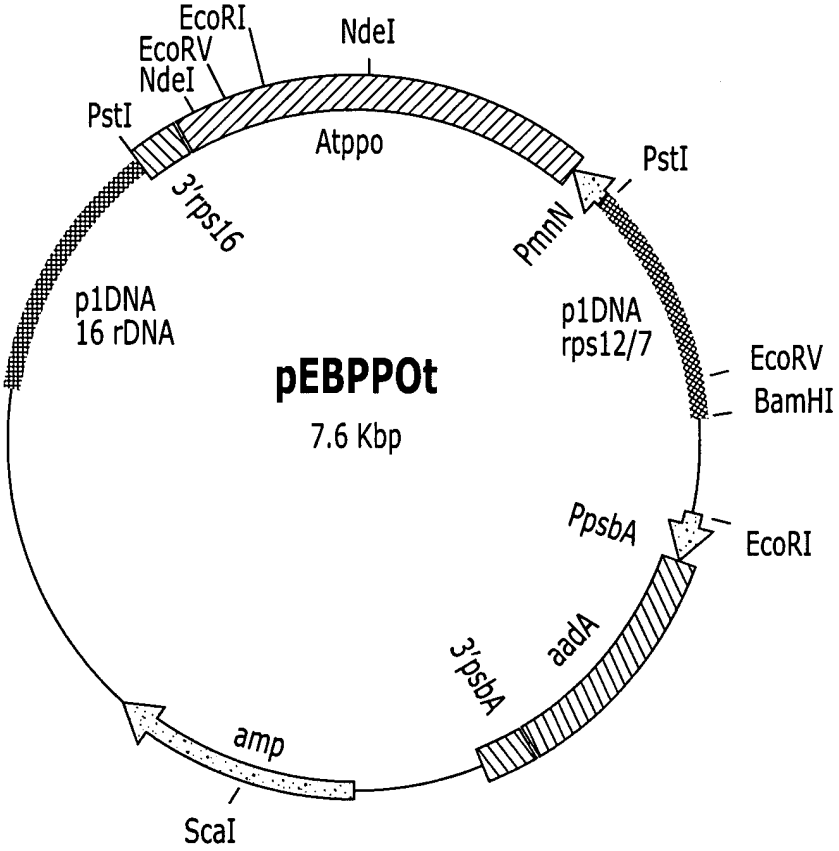

FIG. 1

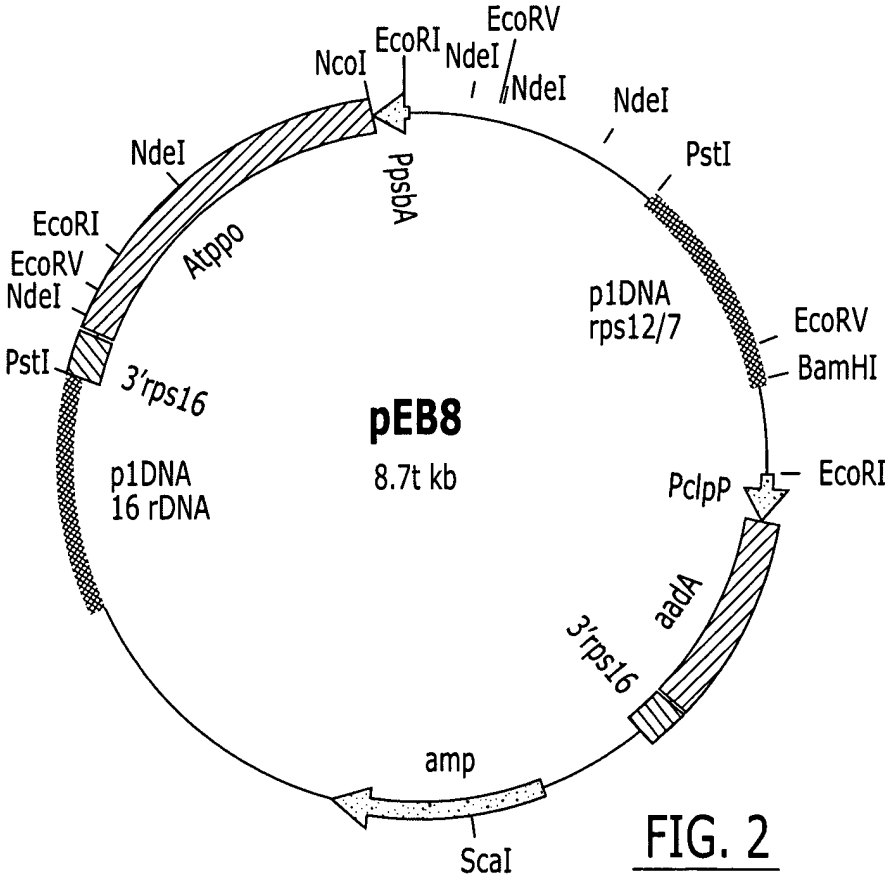
EcoRV
EcoRI
NcoI
NdeI
NdeI
NdeI
PstI
NdeI
PpsbA
EcoRI
EcoRV
NdeI
Atppo
p1DNA
rps12/7
EcoRV
BamHI
PstI
3'rps16
pEB8
8.7t kb
p1DNA
16 rDNA
PclpP
EcoRI
aadA
3'rps16
amp
ScaI

FIG. 2

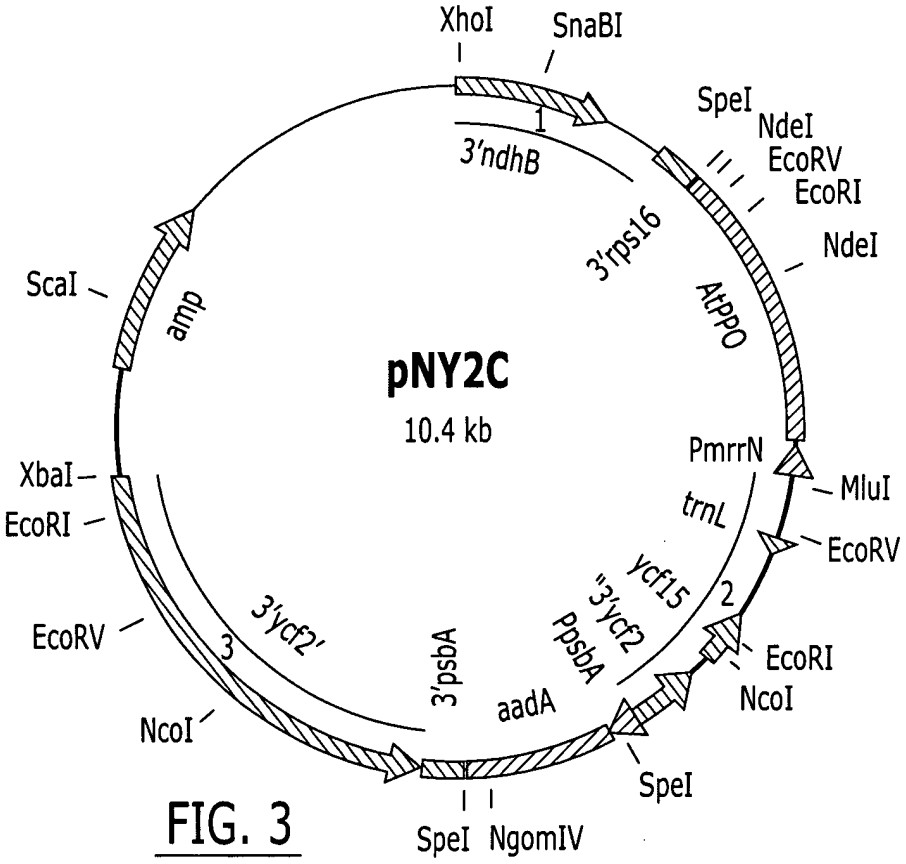
XhoI
SnaBI
SpeI
NdeI
EcoRV
EcoRI
NdeI
MluI
EcoRV
EcoRI
NcoI
SpeI
NgomIV
SpeI
NcoI
EcoRV
EcoRI
XbaI
ScaI
pNY2C
10.4 kb
1
3'ndhB
3'rps16
AtPPO
PmrrN
trnL
2
ycf15
"3'ycf2
PpsbA
aadA
3'psbA
3
3'ycf2'
amp

FIG. 3

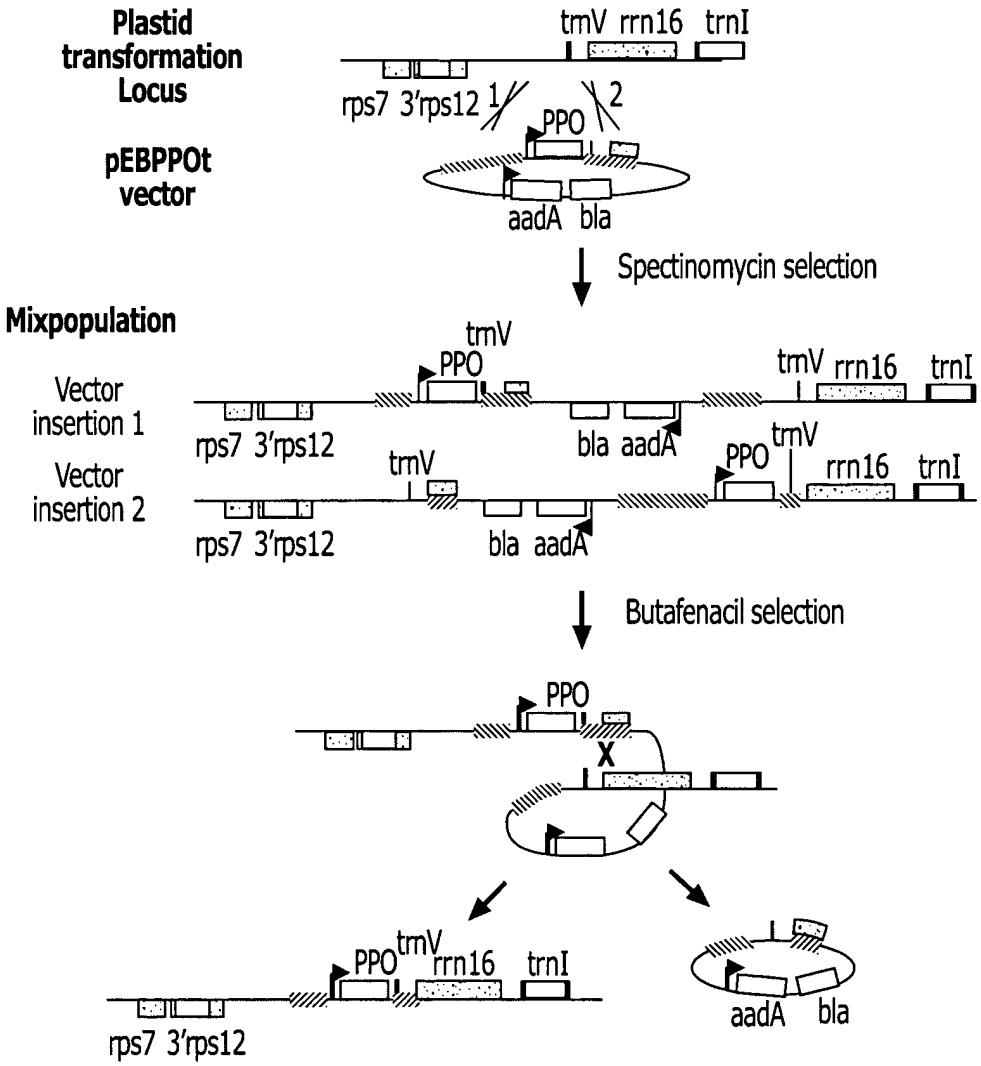
Plastid transformation Locus
trnV rrn16 trnI
rps7 3'rps12
1
2
PPO
pEBPPOt vector
aadA bla
Spectinomycin selection
Mixpopulation
Vector insertion 1
Vector insertion 2
trnV
PPO
trnV rrn16 trnI
rps7 3'rps12
bla aadA
PPO
trnV
rrn16 trnI
trnV
rps7 3'rps12
bla aadA
Butafenacil selection
PPO
X
PPO
trnV
rrn16 trnI
rps7 3'rps12
aadA bla

FIG. 4

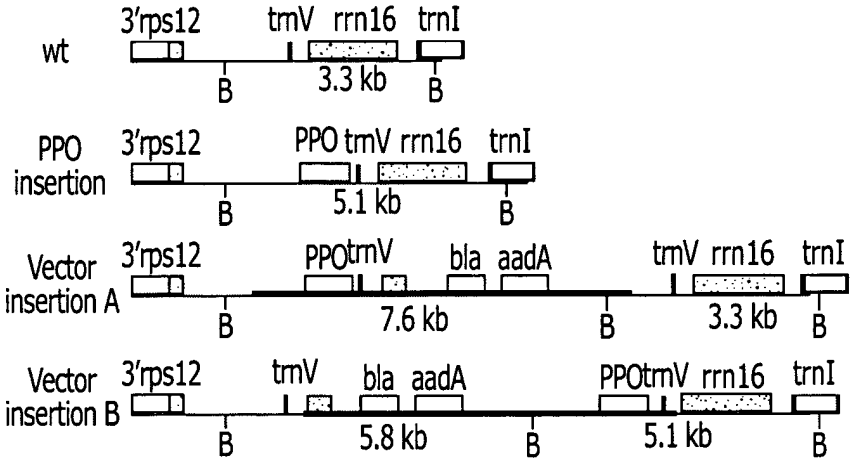
wt
3'rps12
trnV
rrn16
trnI
B
3.3 kb
B
PPO insertion
3'rps12
PPO trnV rrn16
trnI
B
5.1 kb
B
Vector insertion A
3'rps12
PPOtrnV
bla
aadA
trnV rrn16
trnI
B
7.6 kb
B
3.3 kb
B
Vector insertion B
3'rps12
trnV
bla
aadA
PPOtrnV rrn16
trnI
B
5.8 kb
B
5.1 kb
B

FIG. 5A

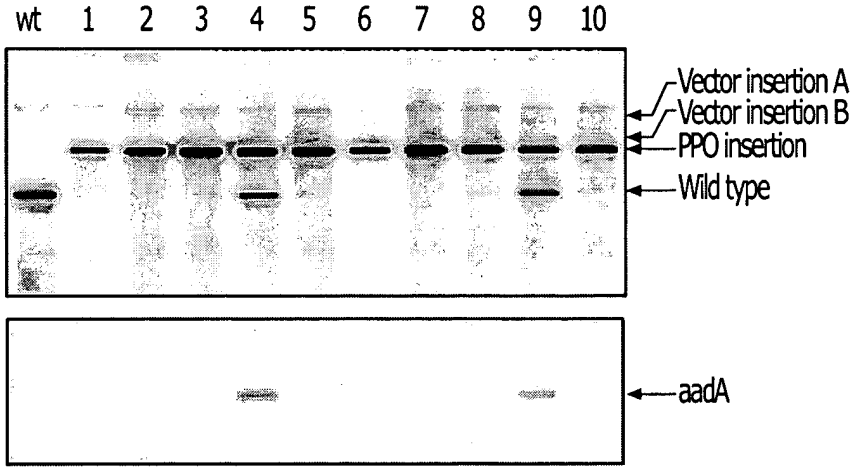
wt
1
2
3
4
5
6
7
8
9
10
Vector insertion A
Vector insertion B
PPO insertion
Wild type
aadA

FIG. 5B

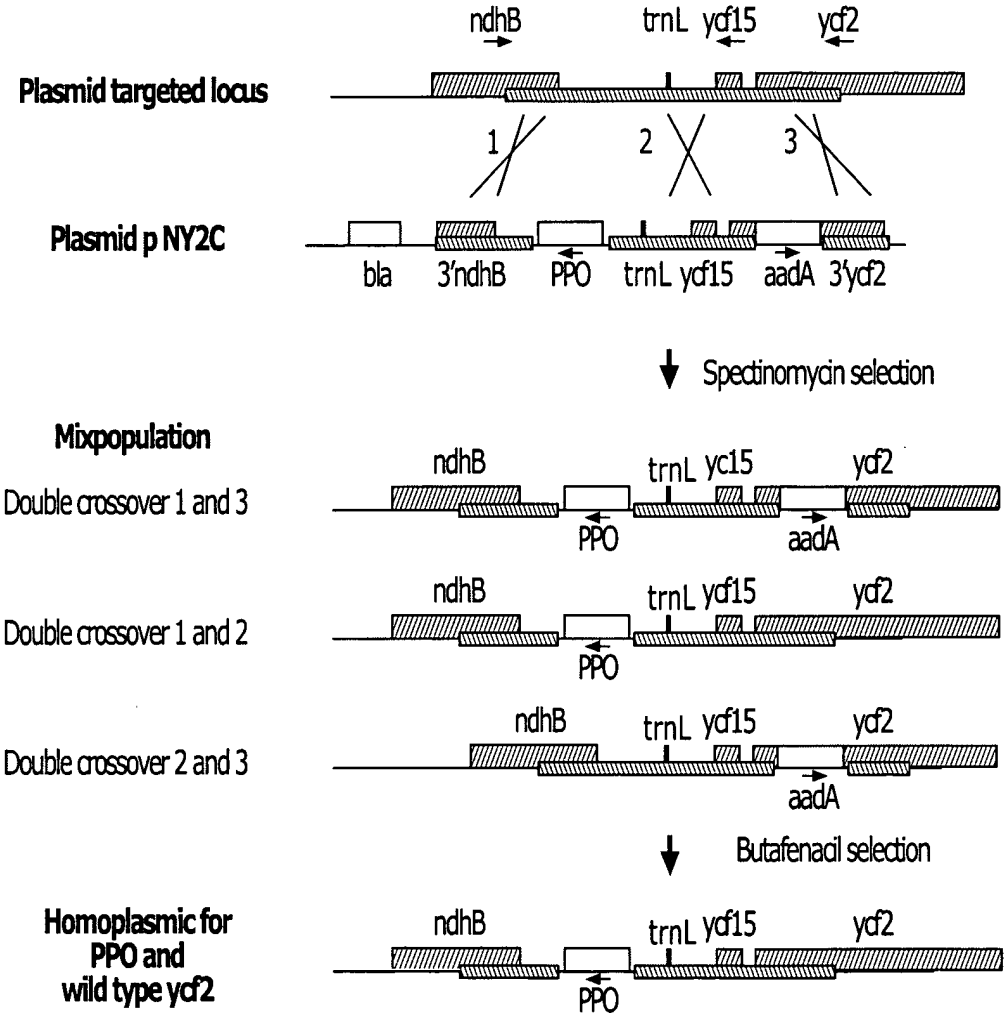
ndhB
trnL
ycf15
ycf2
Plasmid targeted locus
1
2
3
Plasmid p NY2C
bla
3'ndhB
PPO
trnL
ycf15
aadA
3'ycf2
Spectinomycin selection
Mixpopulation
Double crossover 1 and 3
ndhB
trnL
yc15
ycf2
PPO
aadA
Double crossover 1 and 2
ndhB
trnL
ycf15
ycf2
PPO
Double crossover 2 and 3
ndhB
trnL
ycf15
ycf2
aadA
Butafenacil selection
Homoplasmic for PPO and wild type ycf2
ndhB
trnL
ycf15
ycf2
PPO

FIG. 6

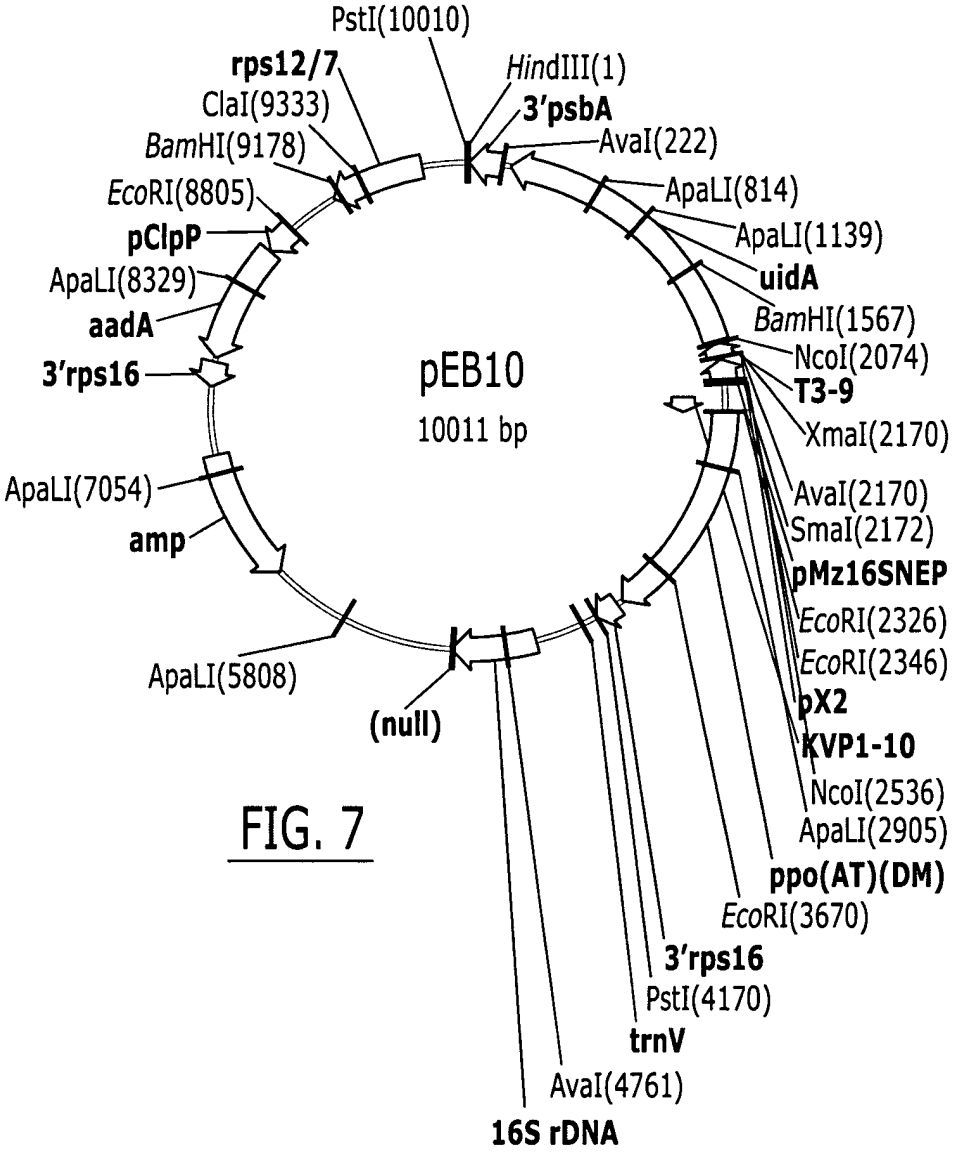
PstI(10010)
rps12/7
ClaI(9333)
BamHI(9178)
EcoRI(8805)
pClpP
ApaLI(8329)
aadA
3'rps16
ApaLI(7054)
amp
ApaLI(5808)
(null)
HindIII(1)
3'psbA
AvaI(222)
ApaLI(814)
ApaLI(1139)
uidA
BamHI(1567)
NcoI(2074)
T3-9
XmaI(2170)
AvaI(2170)
SmaI(2172)
pMz16SNEP
EcoRI(2326)
EcoRI(2346)
pX2
KVP1-10
NcoI(2536)
ApaLI(2905)
ppo(AT)(DM)
EcoRI(3670)
3'rps16
PstI(4170)
trnV
AvaI(4761)
16S rDNA
pEB10
10011 bp

FIG. 7